plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

High genetic diversity declines towards the geographic range periphery of *Adonis vernalis*, a Eurasian dry grassland plant

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Keywords
Abundant centre model; amplified fragment length polymorphism; fragmentation; genetic differentiation; phylogeography; species distribution range.

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Editor
J. Keurentjes

Received: 24 February 2015; Accepted: 24 June 2015

doi:10.1111/plb.12362

ABSTRACT

Genetic diversity is important for species' fitness and evolutionary processes but our knowledge on how it varies across a species' distribution range is limited. The abundant centre hypothesis (ACH) predicts that populations become smaller and more isolated towards the geographic range periphery – a pattern that in turn should be associated with decreasing genetic diversity and increasing genetic differentiation. We tested this hypothesis in *Adonis vernalis*, a dry grassland plant with an extensive Eurasian distribution. Its life-history traits and distribution characteristics suggest a low genetic diversity that decreases and a high genetic differentiation that increases towards the range edge. We analysed AFLP fingerprints in 28 populations along a 4698-km transect from the geographic range core in Russia to the western range periphery in Central and Western Europe. Contrary to our expectation, our analysis revealed high genetic diversity (range of proportion of polymorphic bands = 56–81%, *He* = 0.168–0.238) and low genetic differentiation across populations (Φ_{ST} = 0.18). However, in congruence with the genetic predictions of the ACH, genetic diversity decreased and genetic differentiation increased towards the range periphery. Spanish populations were genetically distinct, suggesting a divergent post-glacial history in this region. The high genetic diversity and low genetic differentiation in the remaining *A. vernalis* populations is surprising given the species' life-history traits and points to the possibility that the species has been widely distributed in the studied region or that it has migrated from a diverse source in an East–West direction, in the past.

INTRODUCTION

Genetic diversity is a fundamental prerequisite for species evolution (Blows & Hoffmann 2005). It regulates the ability of populations to adapt to environmental change, *e.g.* global warming (Thomassen *et al.* 2011) and is an important conservation target (McNeely *et al.* 1990; Bowman 1996; Frankham *et al.* 2010). There is wide consensus that genetic diversity can vary substantially among plant species due to differences in breeding system and life history (Hamrick & Godt 1996). At the intraspecific level, both theory and empirical studies have demonstrated that genetic diversity is influenced by population size, spatial isolation and population dynamics due to the effects of genetic drift, inbreeding, gene flow, founder effects and bottlenecks (Templeton *et al.* 1990; Ellstrand & Elam 1993; Hampe & Petit 2005).

Understanding how genetic diversity varies across species distribution ranges is needed to advance both scientific theory and conservation practice, *i.e.* to explain geographic range limits (Gaston 2009) and to prioritise populations for conservation at a national and regional level (Lesica & Allendorf 1995). However, patterns of genetic diversity across a species distribution range are not well understood. Specifically, it is unclear to what extent populations at the geographic range periphery differ in genetic diversity and differentiation from range-central populations. The abundant centre hypothesis (ACH) predicts that species frequency and abundance declines from the range core to the range periphery in response to decreasing habitat quality and negative population growth (Brown 1984). Such a pattern is also predicted by a more dynamic model, in which bottlenecks and founder effects at range edges play a dominant role (Hampe & Petit 2005). Consequently, genetic diversity should decline and genetic differentiation increase towards the range periphery (Hoffmann & Blows 1994; Vucetich & Waite 2003; Hardie & Hutchings 2010).

Attempts to quantify patterns of genetic diversity and differentiation across a species range have yielded ambiguous results. A meta-analysis by Eckert *et al.* (2008) has shown that 70% of studies confirmed a higher genetic differentiation at the range periphery, while 64% of studies supported the view of low

genetic diversity within peripheral populations. Since then, studies have continued to report divergent results (*e.g.* Hoban *et al.* 2010; Wagner *et al.* 2011). The reason behind the lack of support is not clear, but it has been suggested that life-history traits (Wagner *et al.* 2011), anthropogenic impacts (Wagner *et al.* 2012) and range shift dynamics (Hoban *et al.* 2010) may override the effects of population size and spatial isolation across the range.

In Europe, species at the edge of their distribution range constitute a large proportion of all protected species. For example, in Germany, such peripherally rare species make up 70% (832 species) of all rare plant taxa (Welk 2001). Many of them are widely distributed in regions with a continental climate of Eurasia, and reach their western distribution periphery in the more humid climates of Central Europe (Nosova 1973), where they are rare and often Red-Listed. Due to their threatened status, dry grassland plants in Europe have been studied extensively in the last two decades, *e.g. Astragalus exscapus* (Becker 2003), *Eryngium campestre* (Bylebyl *et al.* 2008), *Iris aphylla* (Wróblewska & Brzosko 2006), *Silene chlorantha* (Lauterbach *et al.* 2011), *S. otites* (Lauterbach *et al.* 2012) and *Stipa capillata* (Hensen *et al.* 2010). Yet, few studies have compared the genetic diversity and differentiation in peripheral dry grassland populations to that found in range-central steppe populations. A study on *Stipa pennata* across Eurasia has found support for the genetic predictions of the ACH (Wagner *et al.* 2012). At the same time, mixed support was found in the congeneric *S. capillata*: while genetic differentiation increased towards the range periphery, genetic diversity did not decrease, possibly due to the species' high selfing rate (Wagner *et al.* 2011).

We studied patterns of genetic diversity and differentiation in populations of *Adonis vernalis* L. (Ranunculaceae), a dry grassland plant species with a Eurasian distribution, along a 4698-km longitudinal transect from Russia to Spain. Its life-history traits (insect pollination and heavy, short distance-dispersed seeds) suggest a generally low population genetic diversity and high genetic differentiation. Furthermore, its distribution patterns are in line with the ACH: at its western periphery, the species occurs in small and isolated dry grasslands. It is Red-Listed in many countries (*e.g.* Austria: Niklfeld & Schratt-Ehrendorfer 1999; Czech Republic: Grulich 2012; Germany: Ludwig & Schnittler 1996; Romania: Dihoru & Dihoru 1994; Ukraine: Didukh 2009; but not in Spain: see Moreno 2008). In Russia, the species is common and not Red-Listed at the federal level (Trutnev 2008; but see Red Lists of some administrative regions, *e.g.* Novikov 2005).

We tested the hypotheses that (i) in general, genetic diversity is low and genetic differentiation high in our study species, but that (ii) genetic diversity declines and genetic differentiation increases from the distribution core to the periphery.

MATERIAL AND METHODS

Study species

Adonis vernalis is an herbaceous perennial hemicryptophyte, characterised by a clumped clonal growth form (Denisow *et al.* 2014). According to Poschkurlat (2000), it can live at least 40–50 years. It can propagate vegetatively, through rhizomes (Ebel & Mühlberg 1987), or generatively by seed (Denisow *et al.* 2014). Flowering of *A. vernalis* occurs between April and June. Its golden-yellow cup-shaped flowers are mainly insect-pollinated, with dipterans, coleopterans, heteroptera and hymenopterans as pollinators (Denisow *et al.* 2014). Although it is self-compatible, seed set in self-pollinated flowers is lower than in open-pollinated flowers (10% *versus* 70%; Denisow *et al.* 2014). The plant produces aggregate fruitlets or achenes (mean weight of a nutlet: 10.6 mg; Klotz *et al.* 2002) with nutrient-rich appendices (elaiosomes) that attract ants. Dispersal is barochorous and myrmecochorous (Fokuhl 2008). Seeds have primary dormancy, need a longer period of cold stratification to germinate (Rouhi *et al.* 2013), and are viable for at least 5 years when stored under laboratory conditions (I. Hensen, unpublished). The species is diploid ($2n = 16$; Marhold *et al.* 2007), and is used in traditional medicine due to its content of cardiac glycosides (Sandberg & Corrigan 2001).

The geographic distribution range of *A. vernalis* stretches over an extensive longitudinal gradient, from Spain in the west to the vicinities of the Yenisei and Lena rivers in Siberia to the east (Fig. 1; Nosova 1973; Poschkurlat 2000). Its latitudinal distribution is bounded by 35°N to the south and 57°N to the north. The species grows in a wide array of habitats, including open forests, forest-steppe, mesic steppe, forest clearings and dry meadows, mostly on calcareous soil (Nosova 1973; Poschkurlat 2000).

Sampling scheme

We collected leaf material in 28 *A. vernalis* populations in six countries (Czech Republic, Germany, Romania, Russia, Spain, Ukraine; Fig. 1). Populations were separated by at least 0.4 km. In each population, we sampled leaf material from 15 individuals within a 30 × 30 m plot. Population size was estimated by counting the approximate number of adult individuals (Table 1).

DNA extraction and amplified fragment length polymorphism (AFLP) analysis

For all following steps, individual samples were arranged at random to prevent artificial differentiation among extraction charges or PCR plates. We applied the same extraction protocol and AFLP procedure as described in Hensen *et al.* (2011), except that, for selective amplification, 32 different primer combinations were tested on 12 samples for their level of variability within and among species, and that four primer combinations were chosen to fingerprint all samples (Table 1). The four combinations were 5′-EcoRI + AAC*FAM-3′/5′-MseI + CCA-3′, 5′-EcoRI + ACT*HEX-3′/5′-MseI + CCA-3′, 5′-EcoRI + AAC*FAM-3′/5′-MseI + CAA-3′ and 5′-EcoRI + ACT*HEX-3′/5′-MseI + CAA-3′. Out of all 420 samples, 59 randomly chosen samples (14.1%) were run repeatedly under the conditions outlined above. Samples that did not produce sufficient electropherogram patterns (*e.g.* smeared and weak bands) were omitted from further analyses. The final dataset consisted of 395 samples (Table 1).

Data analysis

Polymorphic DNA bands were scored as present (1) or absent (0) by using the automatic peak scoring and selec-

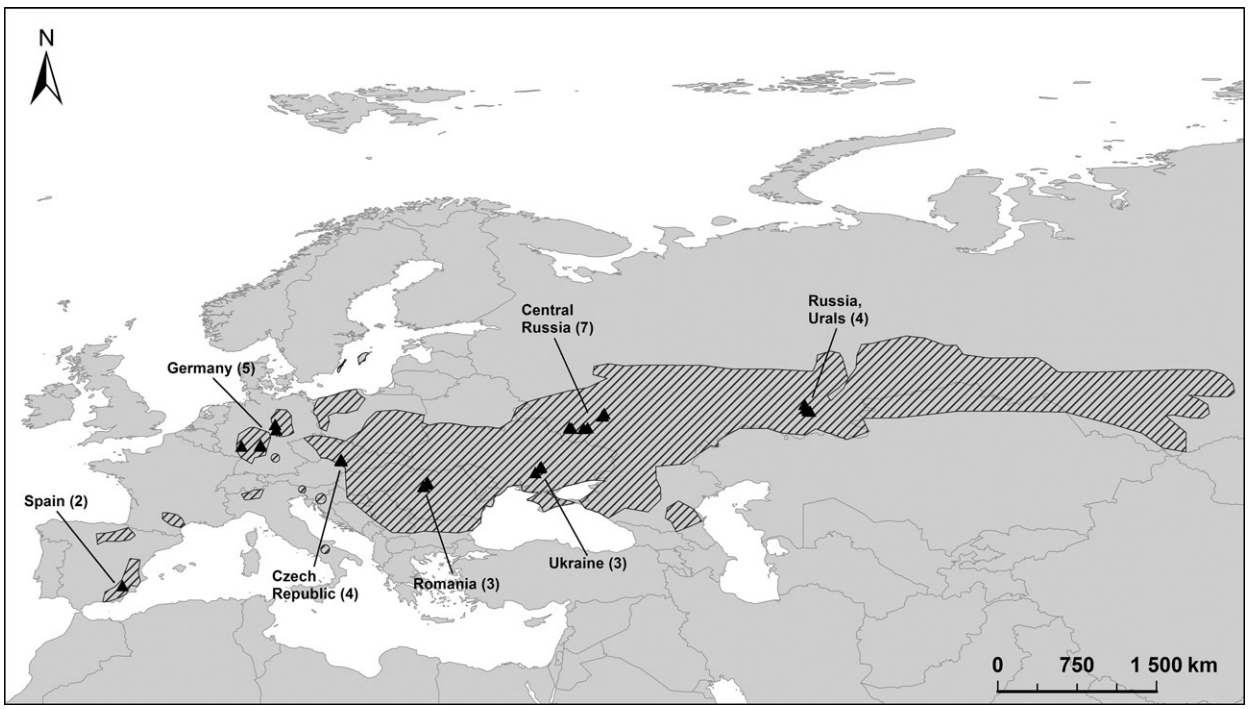

Fig. 1. Geographic distribution range of *Adonis vernalis* (hashed areas; according to Meusel *et al.* 1965). Locations of the study populations are indicated with black triangles. Numbers in parentheses indicate number of study populations in the region. For our analyses, populations from Russia (Urals and central Russia) were considered as central populations, populations from Ukraine and Romania as intermediate populations, and populations from the Czech Republic, Germany and Spain as peripheral populations. See Table 1 for more information.

tion function of the MegaBace Fragment Profiler software (version 1.2; Amersham Biosciences, Freiburg, Germany). We used the following settings: minimum peak height = 20, maximum peak width = 2, minimum size = 50, maximum size = 400, minimum peak–peak distance = 1. Monomorphic peaks were deleted from the output table. To test for the reproducibility of peaks, we used the 59 replicated individuals and followed the protocol of Ley & Hardy (2013) to determine the broad-sense heritability (H^2) and its significance, calculated as F_{ST} (Weir & Cockerham 1984), with the software SPAGeDi (version 1.4; Hardy & Vekemans 2002). Only peaks with $H^2 > 0.5$ and $P < 0.05$ were regarded as reliable, resulting in a final dataset with 95 polymorphic loci, which was used for further analyses.

The proportion of polymorphic bands (PPB), number of private bands and Nei's expected heterozygosity (genetic diversity H_e) were calculated with the software GenAlEx (version 6.501; Peakall & Smouse 2012). We performed ordinary least squares linear regression to test for a relationship between population size and range position (longitude), and genetic diversity and population size. Population size was log-transformed to normalise its distribution. In addition, we also tested linear-straight and hump-backed relationship between genetic diversity and longitude. We checked residual normality by visual inspection. Models were calculated and compared using corrected AIC in R (version 3.1.0; R Core Team 2014).

For the analysis of genetic differentiation, we assigned populations to three geographic regions: central, intermediate and peripheral populations (Fig. 1, Table 1). To inspect relationships among these groups of populations, pair-wise Nei's genetic distances calculated in GenAlEx were used to generate a Neighbor-Net (Bryant & Moulton 2004) with the software SplitsTree (version 4.13.1; Huson & Bryant 2006). Further, an analysis of molecular variance (AMOVA) was applied to investigate the genetic structure and amount of genetic variation within and between populations; Φ-statistics (analogues of F statistics) were extracted and significance levels tested with 999 permutations for each analysis. Populations examined in the AMOVA procedure were assigned to three different groups based on their geographic origin (central, intermediate and peripheral populations; Fig. 1, Table 1). To assess the level of differentiation at different spatial scales, we additionally calculated a separate AMOVA for each of the three regions. AMOVAs were performed in GenAlEx (version 6.501). We used a Mantel test (Mantel 1967) in the R vegan package (version 2.0.-10; Oksanen *et al.* 2013) to test whether genetic (pair-wise Φ_{ST} values) and geographic distances among populations were correlated.

RESULTS

Genetic diversity

Genetic diversity within populations was high. The range of expected heterozygosity (H_e) was 0.168–0.238, while the range of percentage polymorphic bands (PPB) was 55.8–81.1% (Table 1). We detected only two private bands in our dataset (one in population CZ-1 and ESP-2, respectively). Expected heterozygosity showed a hump-backed curve when analysed as

Table 1. Overview of studied *Adonis vernalis* populations. Geographic coordinates in decimal degrees (LAT: latitude; LONG: longitude). N: population size (estimated number of adult individuals), n: number of individuals included in the analysis, H_e: expected heterozygosity, PPB: proportion of polymorphic bands, and Czech R.: Czech Republic.

code	country	LAT	LONG	N	n	H_e	PPB
central populations							
BASH-1	Russia	53.028	56.389	500	15	0.234	72.6
BASH-2	Russia	53.554	56.098	60	15	0.201	65.3
BASH-3	Russia	52.973	56.565	100	15	0.216	64.2
BASH-4	Russia	53.210	56.205	100	15	0.188	66.3
GGO-1	Russia	52.599	38.927	200	14	0.219	68.4
GGO-2	Russia	52.766	39.064	2000	15	0.209	72.6
GGO-3	Russia	52.567	38.891	500	14	0.213	65.3
ZCH-1	Russia	51.532	36.287	200	14	0.202	68.4
ZCH-2	Russia	51.576	36.088	400	14	0.207	65.3
ZCH-3	Russia	51.553	37.653	10,000	13	0.198	64.2
ZCH-4	Russia	51.501	37.309	20,000	15	0.216	72.6
mean						0.209	67.7
intermediate populations							
KKR-1	Ukraine	48.243	33.697	5000	14	0.235	75.8
KKR-2	Ukraine	47.752	33.195	3500	14	0.216	73.7
KKR-3	Ukraine	48.149	33.581	100	14	0.219	68.4
ROM-1	Romania	46.796	23.962	3000	11	0.238	81.1
ROM-2	Romania	46.570	23.677	30	13	0.211	66.3
ROM-3	Romania	46.909	24.041	500	13	0.229	70.5
mean						0.225	72.6
peripheral populations							
CZ-1	Czech R.	48.830	16.637	250	15	0.197	64.2
CZ-2	Czech R.	48.763	16.694	1500	14	0.199	61.1
CZ-3	Czech R.	48.794	16.694	900	14	0.197	66.3
CZ-4	Czech R.	48.849	16.693	3000	13	0.184	60.0
ASCH	Germany	50.004	9.810	3500	15	0.192	60.0
DAR	Germany	50.010	8.210	400	15	0.205	61.1
H	Germany	51.830	11.073	500	15	0.189	63.2
OBER	Germany	50.106	9.823	7000	15	0.179	55.8
UD	Germany	51.386	11.176	35	15	0.168	56.8
ESP-1	Spain	38.144	−1.910	100	12	0.168	63.2
ESP-2	Spain	38.141	−1.911	100	14	0.182	62.1
mean						0.187	61.3
total mean						0.204	66.2

a function of longitude (*t*-test, $P < 0.05$ for slope estimates of the first and second polynomial), with highest values in western Russia that declined towards the range periphery (Fig. 2A; AICc for model with quadratic term: −151.9, without quadratic term: −147.0; intercept only model: −139.4). Expected heterozygosity varied strongly in most eastern populations, *i.e.* those from the Ural Mountains (range of H_e: 0.188–0.234). When these populations were omitted, our analysis fitted a straight line that decreased from the range core to the range periphery (*t*-test, $P < 0.05$ for slope estimate; AICc for model with quadratic term: −131.2, without quadratic term: −131.4; intercept only model: −118.7; Fig. S1A). The same trend was found for the percentage polymorphic bands ($P < 0.05$ for slope estimate; Figs 2B, S1B). However, there was no relationship between expected heterozygosity (H_e) or proportion of polymorphic bands and population size (linear regressions: H_e *versus* population size: $P = 0.629$; PPB *versus* population size: $P = 0.354$ for slope estimates, respectively; Fig. S2).

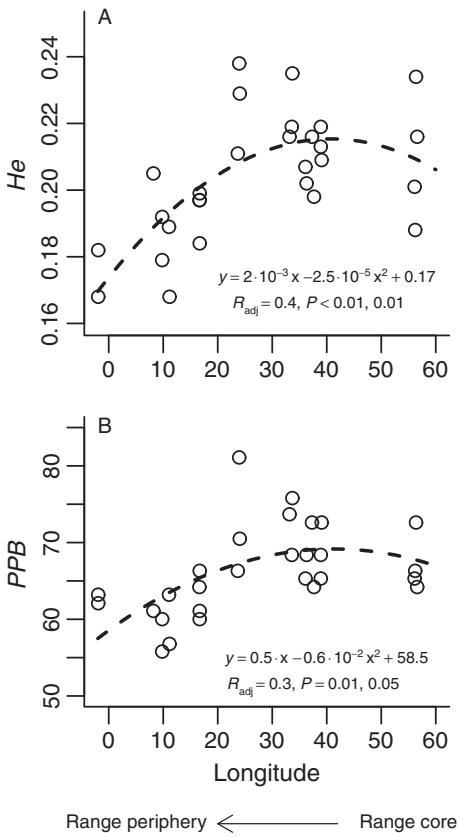

Fig. 2. A: Expected heterozygosity (H_e) and B: proportion of polymorphic bands (PPB) in *Adonis vernalis* populations along a longitudinal transect from the range core to the range periphery. Curves were fitted using linear regression. *P*-values refer to the slope of the first and second term, respectively.

Genetic differentiation

The Neighbor-Net network with all populations revealed sharp partitioning between the two populations from Spain and the remaining population (Fig. 3A). By comparison, the peripheral (without Spain) and central populations were separated to a lesser extent (Fig. 3A). A Neighbor-Net network without the two Spanish populations showed that intermediate populations were arranged between these two groups (Fig. 3B). Moreover, differentiation between peripheral populations was higher than between populations of the central or the intermediate range (Fig. 3B). AMOVA based on the complete dataset (*i.e.* including the populations from Spain) showed that molecular variance was kept mainly within populations (82%) and not among regions (4%) or among populations (14%; Table 2). The Φ_{ST} value of 0.17 demonstrated moderate spatial differentiation. When the three regions were considered separately, molecular variance among populations was also low, but highest for peripheral populations (26% and 14%, with and without populations from Spain, respectively) than for central and intermediate populations (6%, 7%, respectively).

Pair-wise Φ_{ST}-values ranged between −0.02 (converted to zero) (185 km; population GGO-2 and ZCH-4) and 0.26 (2027 km; population GGO-3 and OBER; Table S1) when the

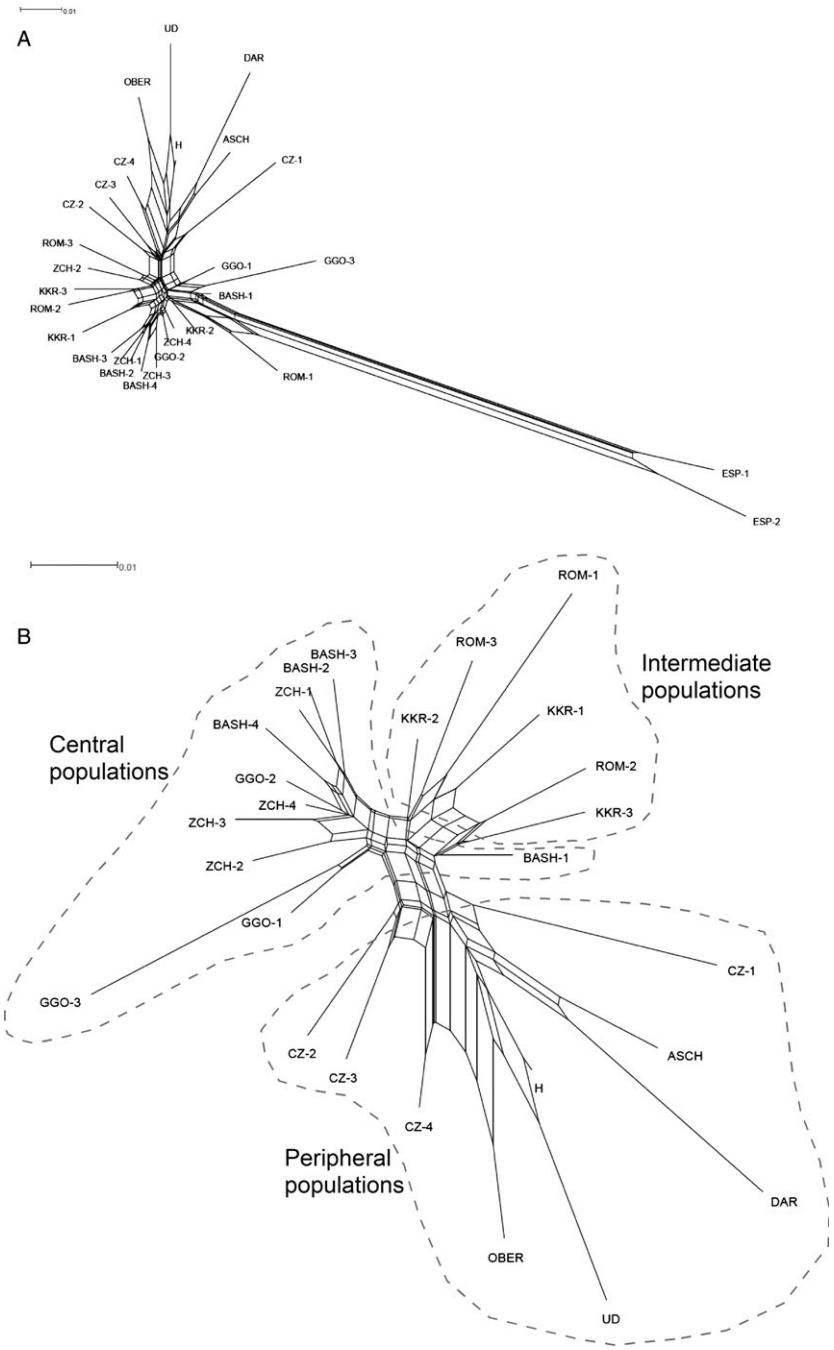

Fig. 3. Neighbor-Net network of *Adonis vernalis* populations based on Nei's pair-wise genetic distances. A: All populations considered. B: Without the two Spanish populations (ESP-1 and ESP-2).

two Spanish populations were not considered, or 0.49 (1795 km, population ESP-2 and UD) when they were considered. We found a significant correlation between genetic distances (pair-wise Φ_{ST}) and geographic distances among populations (Mantel test, $r_M = 0.58$, $P = 0.001$), even when populations from Spain were excluded from the calculations ($r_M = 0.44$, $P = 0.001$; Fig. 4A). However, no significant relationship was found within central ($r_M = 0.001$, $P = 0.449$) and intermediate populations ($r_M = 0.121$, $P = 0.349$), while the relationship remained significant when only peripheral populations were considered ($r_M = 0.942$, $P = 0.001$), even without the Spanish populations ($r_M = 0.357$, $P = 0.003$; Fig. 4B).

DISCUSSION

General patterns of genetic diversity and differentiation

In contrast to our first hypothesis, *A. vernalis* showed surprisingly high within-population genetic diversity across its sampled distribution range and a striking low level of genetic differentiation among populations. Genetic diversity ($H_e = 0.168–0.238$, PPB = 55.8–81.1%) was similar to or higher than the genetic diversity reported from AFLP studies for mixed-mating (*Silene chlorantha*: $H_e = 0.172–0.229$, Lauterbach *et al.* 2011; *Spergularia media*: $H_e = 0.038–0.165$; Prinz *et al.* 2010) and obligate outcrossing species (*Iris*

Table 2. Analysis of molecular variance (AMOVA) among studied *Adonis vernalis* populations, for the complete dataset and for central, intermediate and peripheral populations, respectively. For the peripheral group an additional AMOVA was performed without the populations from Spain since these populations were genetically distinct from the remaining populations within this group. *P*-values are based on 999 permutations.

	df	sum of squares	variance	% of total	*P*-value
complete dataset					
Among regions	2	201.7	0.5	4	<0.001
Among populations	25	957	1.93	14	<0.001
Within populations	367	4055.1	11.05	82	<0.001
central populations					
Among populations	10	226.7	0.77	6	
Within populations	148	1705.7	11.53	94	<0.001
intermediate populations					
Among populations	5	122.9	0.93	7	
Within populations	73	902.6	12.37	93	<0.001
peripheral populations					
Among populations	10	607.5	3.56	26	
Within populations	146	1446.8	9.91	74	<0.001
peripheral populations (without Spain)					
Among populations	8	268	1.62	14	
Within populations	122	1205.9	9.89	86	<0.001

aphylla: H_e = 0.086– 0.131, Wróblewska 2008; *S. otites*: H_e = 0.156–0.24, Lauterbach *et al.* 2012; *Scorzonera purpurea*: H_e = 0.17; Meindl 2011; *Trollius europaeus*: H_e = 0.158–0.229; Després *et al.* 2002; *Ranunculus acris*: H_e = 0.258–0.334; Odat *et al.* 2004). Meyer *et al.* (2015) found high genetic diversity in the congeneric arable weed *A. aestivalis* in Germany (proportion of polymorphic loci = 94–98%). Studies employing other markers revealed similar trends in other *Adonis* species (Boronnikova & Kalendar 2010; Brütting *et al.* 2012). However, given the relatively small sample size within populations, estimates of absolute genetic diversity should be interpreted with caution.

Genetic differentiation was moderate (Φ_{ST} = 0.17) but lower than that reported from AFLP studies for other outcrossing Ranunculaceae, *e.g. Trollius europaeus* (FST = 0.39, max. sampling distance: 3252 km, Després *et al.* 2002), *Ranunculus glacialis* (FST = 0.51, 700 km; Schönswetter *et al.* 2004) and *Anemone shokiana* (GST = 0.38, 300 km; Bian *et al.* 2015). Nevertheless, given the fact that our study covered a larger sampling area than most of the abovementioned studies, our observed Φ_{ST} values are surprising low. For example, pair-wise Φ_{ST} of two populations with a distance of 185 km was zero. Interestingly, relatively low genetic differentiation among (and high genetic diversity within) populations was found in the steppe plant *Pulsatilla vulgaris* (Hensen *et al.* 2005), which is another Ranunculaceae species with similar traits and distribution. Meanwhile, the Mantel test showed that genetic differentiation across the entire dataset was correlated with geographic distance. This demonstrates that genetic differentiation in *A. vernalis* was mediated by an isolation-by-distance mechanism. In addition, the relatively high Φ_{ST} variation across geographic distances indicates that genetic drift was also an important factor in shaping genetic distance (Hutchison & Templeton 1999).

Although high genetic diversity and pronounced genetic differentiation is typical of outcrossing species (Hamrick & Godt 1996), our observed values are striking given the species' limited dispersal ability and the fact that many populations are small and fragmented. In view of the species' heavy myrmeco-

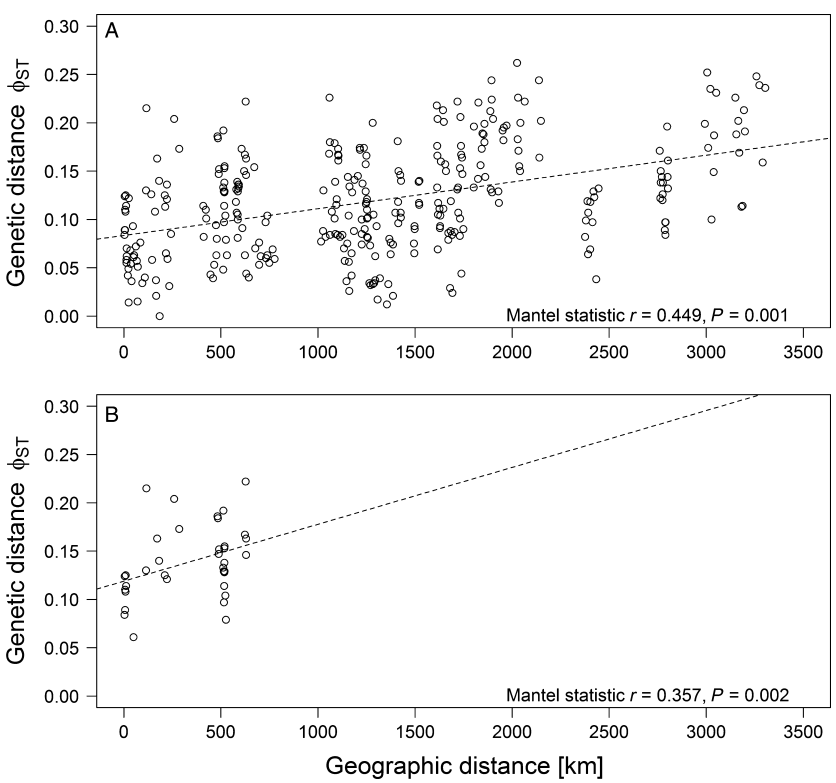

Fig. 4. Correlation between genetic and geographic distances among the investigated *Adonis vernalis* populations. A: complete dataset without Spanish populations; B: peripheral populations without Spanish populations.

chorous and barochorous seeds, it is likely that pollen dispersal rather than seed dispersal mediated most of the gene flow (Fenster 1991). However, gene flow *via* long-distance pollen cannot account for the observed pattern given that bumblebees can only fly short distances (max. 2.5 km) and have small foraging ranges (Hagen *et al.* 2011). The almost complete lack of private loci speaks in favour of a more continuous distribution in the past. The species' broad habitat niche, perennial life form, ability to propagate vegetatively and poor self-compatibility could have decelerated genetic drift and inbreeding and allowed it to maintain a genetic network of regional meta-populations since then.

Core–periphery gradient

Our study found that genetic diversity decreased and genetic differentiation increased from the range core to the range periphery. A similar pattern was found for the steppe species *Stipa pennata*, across the western half of its distribution range (Wagner *et al.* 2012). An increase in genetic differentiation towards the range periphery was also found for the steppe plants *Iris aphylla* (Wróblewska 2008) and *Stipa capillata* (Wagner *et al.* 2011). Consequently, our findings support the genetic predictions of the abundant centre hypothesis and suggest that small population size and spatial isolation have accelerated genetic drift, inbreeding and have hampered gene flow at the range periphery.

Our understanding of the migration history of Eurasian steppe plants is still scarce (Hensen *et al.* 2005, 2010; Wróblewska 2008) but our results suggest that the peripheral, intermediate and central *A. vernalis* populations could have formed a more continuous distribution in the past. Evidence that some parts of Europe were never covered by continuous forests since the end of the Pleistocene supports this assumption (*e.g.* Feurdean *et al.* 2015; Pokorný *et al.* 2015). For example, Magyari *et al.* (2010) identified wooded steppe, which represents a highly suitable habitat for *A. vernalis*, as the main vegetation type in the Hungarian Plain during the first half of the Holocence. Further, palaeo-environmental data of the Pannonian Plain provided evidence that about 20% of the landscape were characterised by a stable openness during the entire Holocene, and that Neolithic people contributed to maintain these existing open habitats (Kuneš *et al.* 2015). The presence of open landscapes in the Middle Ages is also well documented, both in Central Europe (Poschlod & WallisDeVries 2002) and in Russia (Chibilyev 1998). Consequently, such a continuous distribution could have existed in the Middle Ages, when traditional pastoralism, hay-making and logging created an open landscape in Europe. The subsequent change in land use could have reduced the western distribution of *A. vernalis*, leaving small and isolated rear edge populations. However, given the almost complete lack of private alleles among peripheral populations, our results suggest that peripheral populations have either not been isolated for a very long time or that they form a 'leading edge' (Hampe & Petit 2005). In the latter case, our results could rather reflect the species advance from the distribution core in Russia to the periphery in Central Europe, as suggested by Wróblewska (2008) for the steppe plant *Iris aphylla* and by Šmídová *et al.* (2011) for the Eurasian wetland plant *Ligularia sibirica*.

Interestingly, genetic distance between peripheral Iberian and Central European populations was the highest in the entire dataset (Φ_{ST} = 0.372–0.493). The Neighbor-Net network demonstrated that it was even higher than the genetic distance between Central European and Russian populations, despite a similar spatial distance. This result implies that gene flow between Iberian and Central European peripheral populations must have been weak in the past. It is possible that these two groups split as early as in the Early Holocene, when forests replaced steppe across Europe (Adams & Faure 1997). However, the small number of studied populations in Spain limits our interpretation and calls for more comprehensive studies.

ACKNOWLEDGEMENTS

We thank Ivan Moysienko, Vasiliy Kucherevskiy and staff of the Krivorozhskiy Botanical Garden (Ukraine), Vasiliy Martynenko, Liliya Sultangareeva, Rafael Sultangareev, Larisa Skolzneva, Nikolay Skolznev, Andrey Vlasov; staff of the Zapovednik Galichya Gora, the Tsentral'no-Chernozemnyy Zapovednik, the Bashkiriya National Park (Russia) and the Environment Agency of the Autonomous Community Murcia (Spain) for support during fieldwork. Birgit Müller and Antje Dippel (Germany) provided assistance during lab work. This work was supported by a travel grant to VW from the German Academic Exchange Service (DAAD), and the long-term research development project of the Institute of Botany, Czech Academy of Sciences (RVO 67985939) to JD.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) Expected heterozygosity (H_e) and (B) proportion of polymorphic bands (PPB) in *Adonis vernalis* populations along a longitudinal transect from the range core to the range periphery (Ural populations omitted).

Figure S2. (A) Expected heterozygosity (H_e) and (B) proportion of polymorphic bands (PPB) in *Adonis vernalis* populations as a function of population size.

Table S1. Pair-wise Φ_{ST} values (lower triangle) between the investigated *Adonis vernalis* populations. The upper triangle represents the probability of the corresponding pair-wise Φ_{ST} values (**$P < 0.05$; NS not significant).

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